

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Chien-Chung Cheng et al Art Unit :
Serial No. : Examiner :
Filed : January 2, 2002
Title : METHOD OF CLEAVING A NUCLEIC ACID BULGE

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the title:

Replace the title of the application with the following:
-- NUCLEIC ACID BULGE-DETECTING AGENT --

In the specification:

Insert the following paragraph beginning at page 1, line 1:

-- This application is a divisional application of U.S. Serial Number 09/302,334 filed April 30, 1999, pending issue. --

Replace the paragraph beginning at page 7, line 18 with the following rewritten paragraph:

-- Note that the metal ion of each of the complexes of formula (I) adopts an octahedral coordination. For example, the X-ray crystal structure of cobalt(II)(hexaazacyclo-

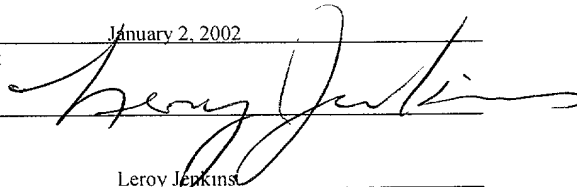
CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No EL932075795US

I hereby certify under 37 CFR §1 10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C 20231

Date of Deposit January 2, 2002

Signature



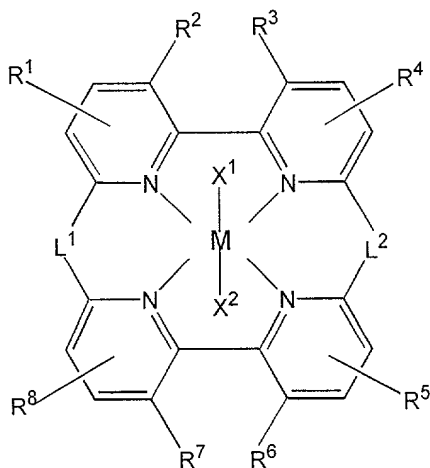
Typed or Printed Name of Person Signing Certificate

In the claims:

Claims 17-27 have been cancelled.

Claims 1-16 have been reiterated as follows:

1. (Reiterated) A metal complex of the following formula:



wherein

each of R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , and R^8 , independently, is hydrogen, alkyl, alkoxy, hydroxyl, hydroxylalkyl, halo, haloalkyl, amino, aminoalkyl, alkylcarbonylamino, alkylaminocarbonyl, alkylcarbonyl, alkylcarbonylalkyl, alkoxycarbonyl, alkylcarbonyloxy, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; each of R^2 and R^3 , and R^6 and R^7 , independently, optionally joining together to form a cyclic moiety fused with the two pyridyl rings to which R^2 and R^3 , or R^6 and R^7 are bonded; the cyclic moiety, if present, optionally being substituted with alkyl, alkoxy, hydroxyl, hydroxylalkyl, halo, haloalkyl, amino, aminoalkyl, alkylcarbonylamino, alkylaminocarbonyl, alkylcarbonyl, alkylcarbonylalkyl, alkoxycarbonyl, alkylcarbonyloxy, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

each of L^1 and L^2 , independently, is $-C(R^a)(R^b)-$, $-O-$, $-S-$, or $-N(R^c)-$; each of R^a , R^b , and R^c , independently, is hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

M is a Co, Ni, Ru, Rh, Mn, Os, Ag, Cr, Zn, Cd, Hg, Re, Ir, Pt, or Pd ion; and

each of X^1 and X^2 , independently, is a labile ligand;

or a salt thereof.

phane)(trifluoroacetate)₂, i.e., Co^{II}(HAPP)(TFA)₂, reveals that the complex contains two labile axial TFA ligands, and two linked 1,10-phenanthroline moieties where all four pyridyl nitrogen atoms are locating on the same coordination plane. The average Co-N distance is approximately 1.86 Å. EPR spectrum of the Co^{II} complex gave a g_{av} value at 2.005-2.331 in methanol, indicating the presence of an octahedral Co^{II} complex. When one equivalent of pyridine was added, it rapidly displaced one of the axial TFA ligands under ambient conditions, as monitored by EPR spectroscopy, suggesting that the TFA ligands are labile. The TFA ligands can also be readily substituted by water upon dissolution of the complex in aqueous buffer. --

Replace the paragraph beginning at page 13, line 2 with the following rewritten paragraph:

-- A 26-mer 5'-GCAGACTGAGCCTGGGAGCTCTCTGC-3' (SEQ ID No. 4) (D, Figure 1) was used as the DNA substrate. It was prepared according to the same procedures as described in Example 1. Note that substrate D only differs from substrate A in that its bulge contains one less base. --

Replace the paragraph beginning at page 13, line 13 with the following rewritten paragraph:

-- Co^{II}(HAPP)(TFA)₂ (0.6 μM) was allowed to react under identical conditions as described above with a single-stranded 16-mer of the sequence 5'-GCCAGATCTGAGCCTG-3' (SEQ ID No. 2) (B, Figure 1) in the presence of H₂O₂. No specific cleavage was observed at the 5'-TCT-3' site, even when the concentration of the cobalt complex was increased by 20-fold. The single-stranded substrate was then allowed to anneal with a complementary DNA strand 5'-CAGGGCTCTCTGCC-3' (SEQ ID No. 3) to form a double-stranded DNA with a three-base bulge (C, Figure 1). When the Co^{II} complex was added to the double-stranded substrate, enhanced DNA cleavage was observed at the 5'-TCT-3' bulge. These results indicate that the Co^{II} complex serves as a DNA bulge-specific cleavage reagent without significant specificity towards the corresponding sequence in the single-stranded DNA. --

2. (Reiterated) The metal complex of claim 1, wherein each of R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , and R^8 , independently, is hydrogen, alkyl, or alkoxy.

3. (Reiterated) The metal complex of claim 1, wherein each of R^2 and R^3 , and R^6 and R^7 , independently, join together to form a cyclic moiety; the cyclic moiety being benzene.

4. (Reiterated) The metal complex of claim 3, wherein the cyclic moiety is unsubstituted.

5. (Reiterated) The metal complex of claim 4, wherein each of R^1 , R^4 , R^5 , and R^8 , independently, is hydrogen, alkyl, or alkoxy.

6. (Reiterated) The metal complex of claim 5, wherein each of R^1 , R^4 , R^5 , and R^8 , independently, is hydrogen.

7. (Reiterated) The metal complex of claim 6, wherein each of L^1 and L^2 , independently, is $-N(R^c)-$ where R^c is hydrogen.

8. (Reiterated) The metal complex of claim 7, wherein M is Co.

9. (Reiterated) The metal complex of claim 8, wherein X^1 and X^2 , independently, is trifluoroacetate.

10. (Reiterated) The metal complex of claim 9, wherein said complex is cobalt(II) (hexaazacyclophane) (trifluoroacetate).

11. (Reiterated) The metal complex of claim 1, wherein each of L^1 and L^2 , independently, is $-S-$ or $-N(R^c)-$.

12. (Reiterated) The metal complex of claim 11, wherein each of L^1 and L^2 , independently, is $-N(R^c)-$ where R^c is hydrogen.
13. (Reiterated) The metal complex of claim 1, wherein M is Co, Ru, or Mn.
14. (Reiterated) The metal complex of claim 13, wherein M is Co.
15. (Reiterated) The metal complex of claim 1, wherein X^1 and X^2 , independently, is H_2O , Cl, trifluoroacetate, or pyridine.
16. (Reiterated) The metal complex of claim 15, wherein X^1 and X^2 , independently, is trifluoroacetate.

Applicant : Cheng et al
Serial No. :
Filed : January 2, 2002
Page : 6

Attorney's Docket No.: 08919-018002 / 03A-871028
(DIV)

REMARKS

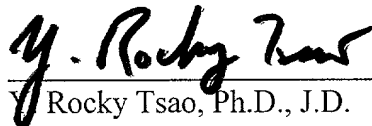
Applicants have amended the Specification to incorporate amendments that have been made to the parent application. Prompt examination of the present application, as amended, is respectfully requested.

Attached is a marked-up version of the changes being made by the current amendment.

Please apply any other charges to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 1-2-02


Rocky Tsao, Ph.D., J.D.
Attorney for Applicants
Reg. No. 34,053

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

20353063.doc

20353063.doc

Version with markings to show changes made

In the title:

Replace the title of the application with the following:

[METHOD OF CLEAVING A NUCLEIC ACID BULGE] NUCLEIC ACID BULGE-
DETECTING AGENT

In the specification:

Insert the following paragraph beginning at page 1, line 1:

This application is a divisional application of U.S. Serial Number 09/302,334 filed April 30, 1999, pending issue.

Replace the paragraph beginning at page 7, line 18 with the following rewritten paragraph:

Note that the metal ion of each of the complexes of formula (I) adopts an [octehedral] octahedral coordination. For example, the X-ray crystal structure of cobalt(II)(hexaazacyclophane)(trifluoroacetate)₂, i.e., Co^{II}(HAPP)(TFA)₂, reveals that the complex contains two labile axial TFA ligands, and two linked 1,10-phenanthroline moieties where all four pyridyl nitrogen atoms are locating on the same coordination plane. The average Co-N distance is approximately 1.86 Å. EPR spectrum of the Co^{II} complex gave a g_{av} value at 2.005-2.331 in methanol, indicating the presence of an octahedral Co^{II} complex. When one equivalent of pyridine was added, it rapidly displaced one of the axial TFA ligands under ambient conditions, as monitored by EPR spectroscopy, suggesting that the TFA ligands are labile. The TFA ligands can also be readily substituted by water upon dissolution of the complex in aqueous buffer.

Also replace the paragraph beginning at page 13, line 2 with the following rewritten paragraph:

A 26-mer 5'-GCAGACTGAGCCTGGGAGCTCTCTGC-3' (SEQ ID No. 4) (D, Figure 1) was used as the DNA substrate. It was prepared according to the same procedures as described

002019-018002

in Example 1. Note that substrate D only differs from substrate A in that its bulge contains one less base.

Further replace the paragraph beginning at page 13, line 13 with the following rewritten paragraph:

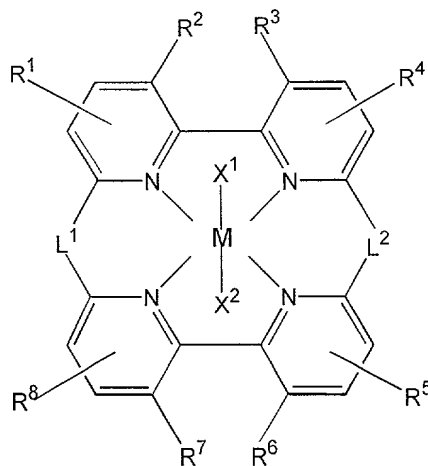
$\text{Co}^{\text{II}}(\text{HAPP})(\text{TFA})_2$ (0.6 μM) was allowed to react under identical conditions as described above with a single-stranded 16-mer of the sequence 5'-GCCAGATCTGAGCCTG-3' (SEQ ID No. 2) (B, Figure 1) in the presence of H_2O_2 . No specific cleavage was observed at the 5'-TCT-3' site, even when the concentration of the cobalt complex was increased by 20-fold. The single-stranded substrate was then allowed to anneal with a complementary DNA strand 5'-CAGGGCTCTCTGCC-3' (SEQ ID No. 3) to form a double-stranded DNA with a three-base bulge (C, Figure 1). When the Co^{II} complex was added to the double-stranded substrate, enhanced DNA cleavage was observed at the 5'-TCT-3' bulge. These results indicate that the Co^{II} complex serves as a DNA bulge-specific cleavage reagent without significant specificity towards the corresponding sequence in the single-stranded DNA.

In the claims:

Claims 17-27 have been cancelled.

Claims 1-16 have been reiterated as follows:

1. (Reiterated) A metal complex of the following formula:



wherein

each of R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , and R^8 , independently, is hydrogen, alkyl, alkoxy, hydroxyl, hydroxylalkyl, halo, haloalkyl, amino, aminoalkyl, alkylcarbonylamino, alkylaminocarbonyl, alkylcarbonyl, alkylcarbonylalkyl, alkoxycarbonyl, alkylcarbonyloxy, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; each of R^2 and R^3 , and R^6 and R^7 , independently, optionally joining together to form a cyclic moiety fused with the two pyridyl rings to which R^2 and R^3 , or R^6 and R^7 are bonded; the cyclic moiety, if present, optionally being substituted with alkyl, alkoxy, hydroxyl, hydroxylalkyl, halo, haloalkyl, amino, aminoalkyl, alkylcarbonylamino, alkylaminocarbonyl, alkylcarbonyl, alkylcarbonylalkyl, alkoxycarbonyl, alkylcarbonyloxy, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

each of L^1 and L^2 , independently, is $-C(R^a)(R^b)-$, $-O-$, $-S-$, or $-N(R^c)-$; each of R^a , R^b , and R^c , independently, is hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

M is a Co, Ni, Ru, Rh, Mn, Os, Ag, Cr, Zn, Cd, Hg, Re, Ir, Pt, or Pd ion; and

each of X^1 and X^2 , independently, is a labile ligand;

or a salt thereof.

2. (Reiterated) The metal complex of claim 1, wherein each of R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , and R^8 , independently, is hydrogen, alkyl, or alkoxy.

3. (Reiterated) The metal complex of claim 1, wherein each of R^2 and R^3 , and R^6 and R^7 , independently, join together to form a cyclic moiety; the cyclic moiety being benzene.

4. (Reiterated) The metal complex of claim 3, wherein the cyclic moiety is unsubstituted.

5. (Reiterated) The metal complex of claim 4, wherein each of R^1 , R^4 , R^5 , and R^8 , independently, is hydrogen, alkyl, or alkoxy.

6. (Reiterated) The metal complex of claim 5, wherein each of R^1 , R^4 , R^5 , and R^8 , independently, is hydrogen.

7. (Reiterated) The metal complex of claim 6, wherein each of L^1 and L^2 , independently, is $-N(R^c)-$ where R^c is hydrogen.

8. (Reiterated) The metal complex of claim 7, wherein M is Co.

9. (Reiterated) The metal complex of claim 8, wherein X^1 and X^2 , independently, is trifluoroacetate.

10. (Reiterated) The metal complex of claim 9, wherein said complex is cobalt(II) (hexaazacyclophane) (trifluoroacetate).

11. (Reiterated) The metal complex of claim 1, wherein each of L^1 and L^2 , independently, is $-S-$ or $-N(R^c)-$.

12. (Reiterated) The metal complex of claim 11, wherein each of L^1 and L^2 , independently, is $-N(R^c)-$ where R^c is hydrogen.

13. (Reiterated) The metal complex of claim 1, wherein M is Co, Ru, or Mn.

14. (Reiterated) The metal complex of claim 13, wherein M is Co.

15. (Reiterated) The metal complex of claim 1, wherein X^1 and X^2 , independently, is H_2O , Cl, trifluoroacetate, or pyridine.

16. (Reiterated) The metal complex of claim 15, wherein X^1 and X^2 , independently, is trifluoroacetate.

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD OF CLEAVING A NUCLEIC ACID BULGE, the specification of which:

[X] is attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Serial No.	Filing Date	Status
09/302,334	April 30, 1999	Allowed

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Y. Rocky Tsao, Reg. 34,053
John F. Hayden, Reg. 37,640
Jeffrey D. Hsi, Reg. No. 40,024

Eric Prael, Reg. 32,590
Frank R. Occhiuti, Reg. 35,306
Harold H. Fox, Reg. No. 41,498

Address all telephone calls to Y. ROCKY TSAO at telephone number (617) 542-5070.

Address all correspondence to Y. ROCKY TSAO at:

FISH & RICHARDSON P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name of Inventor: CHIEN-CHUNG CHENG

Inventor's Signature:

Chien Chung Cheng

Date:

Dec. 19, 2001

Residence Address:

Taipei, Taiwan

Citizenship:

Taiwan, R.O.C.

Post Office Address:

4F-1, No. 8, Ln. 55, Hulin Street
Taipei, Taiwan

Combined Declaration and Power of Attorney

Page 2 of 2 Pages

Full Name of Inventor: WEN JWU WANG

Inventor's Signature:

Wang
Taiwan

Date:

Dec. 19, 2001

Residence Address:

Taipei, Taiwan

Citizenship:

Taiwan, R.O.C.

Post Office Address:

2F, No. 15, Ln. 328, Chung-Cheng Road
Hsin, Tien, Taipei, Taiwan

20353099.doc

[illegible]